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Tolerance to Intravenous Nitroglycerin in Patients With Congestive Heart Failure: Role of Increased Intravascular Volume, Neurohumoral Activation and Lack of Prevention With N-Acetylcysteine

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To better understand the mechanism of nitrate tolerance in patients with congestive heart failure, 13 patients received a 24 h infusion of nitroglycerin (1.5 μg/kg body weight per min) with or without N-acetylcysteine (225 mg/kg per 24 h). The infusions were separated by a 24 h nitrate-free interval. By the end of the nitroglycerin infusion, mean arterial pressure had returned to baseline values and there was a significant increase in ventricular filling pressures and systemic vascular resistance compared with values after 1 h of treatment. The simultaneous infusion of N-acetylcysteine had no effect on these changes.

Although a strict fluid restriction of 1.5 liters/day was maintained for 1 week before and throughout the study, after 24 h of nitroglycerin infusion there was a significant and similar degree of hemodilution whether nitroglycerin was infused alone (9.1 ± 4.3%) or with N-acetylcysteine (8.7 ± 4.1%). This hemodilution corresponded to an increase in intravascular volume of 745 ± 382 ml, most of which occurred during the 1st h. Plasma renin activity increased and plasma atrial natriuretic peptide decreased during the infusion.

The results of this study suggest that nitrate tolerance is multifactorial. In addition to the previously described pharmacologic tolerance to the effect of nitroglycerin on vascular smooth muscle, a capillary fluid shift from the extravascular to intravascular space appears to be involved, especially during the 1st h of the infusion. A third mechanism, reflex neurohumoral activation, also seems to contribute to the genesis of nitroglycerin tolerance.

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Nitrates have been used successfully in the treatment of angina (1,2) and more recently to improve ventricular function in patients with congestive heart failure (3–6). Unfortunately, when they are given without interruption, a certain degree of tolerance to their beneficial effects appears within 24 h (1,2,5–10). The most important mechanism by which nitrate tolerance occurs appears to be a loss of its direct pharmacologic vasodilating properties (1). One theory advanced to explain this tolerance is sulfhydryl group depletion (5–14). More recent studies (15–17) have questioned this mechanism and proposed that tolerance is the result of a change in affinity of the intracellular transformation site for organic nitrates. Two nonpharmacologic causes of nitrate tolerance have also been proposed: reflex neurohumoral activation (5,6,18) and intravascular volume expansion (19–22).

In this study, we examined 1) whether a continuous infusion of N-acetylcysteine in doses comparable with those used in the treatment of acetaminophen intoxication could prevent nitrate tolerance; 2) the relation of changes in intravascular volume to the development of hemodynamic tolerance to nitroglycerin; and 3) the role of neurohumoral activation in the development of hemodynamic tolerance to nitroglycerin.

Methods

Study patients. Thirteen men (aged 43 to 72 years, mean 61) were studied. All had severe chronic congestive heart failure (New York Heart Association functional class IV) caused by ischemic heart disease. No patient had had an acute myocardial infarction or unstable angina in the previ-

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ous 6 months. The patients were admitted and stabilized in hospital for a minimum of 5 days on a 2 g sodium diet and 1.5 liters fluid restriction per day. All patients were at bed rest. All vasodilators were discontinued a minimum of 5 days before the study, and patients were maintained on digoxin and diuretic drugs. Once considered maximally controlled with <0.5 kg weight change over 3 consecutive days, they were transferred to the coronary care unit. Patients were maintained on a 2 g sodium diet and 1.5 liters fluid restriction and were weighed daily throughout the study. The 1.5 liter fluid restriction included both oral and intravenous liquids such that when intravenous liquids were given, oral intake was reduced correspondingly. Written informed consent was obtained from every patient. All study procedures were approved by the Ethics Committee of Hôpital du Sacré-Coeur de Montreal on March 21, 1988.

Preinfusion measurements. The evening before the study (23), a 7F triple lumen, flow-directed, balloon-tipped thermodilution catheter (Edwards Laboratories) was inserted transcutaneously through the right internal jugular vein by the Seldinger technique and advanced to a pulmonary wedge position according to pressure tracings. Pulmonary artery, right atrial and pulmonary capillary wedge pressures were monitored using a Gould-Statham P2311d transducer and were recorded on an Electronics for Medicine VR-6 photographic recorder. Cardiac output was measured in triplicate by means of the computerized thermodilution technique by injecting 10 ml iced 5% dextrose in water. Systemic arterial blood pressure was measured through a radial cannula. All derived hemodynamic variables were calculated as previously described (24).

The next morning, while the patient was still fasting and before being given any medication, a sample of blood was drawn, labeled with chromium and reinjected into the patient to measure red blood cell and intravascular volume. After 30 min of equilibration, baseline hemodynamic measurements were obtained and blood was drawn from the arterial cannula for measurement of red blood cell volume, hematocrit and hemoglobin, creatinine, blood urea nitrogen and plasma neurohumoral levels (renin, aldosterone, arginine vasopressin, atrial natriuretic peptide, norepinephrine and epinephrine).

Infusion protocol. Patients were then randomly assigned to receive either nitroglycerin alone or nitroglycerin plus N-acetylcysteine. When patients were given nitroglycerin alone, the drug was started at 0.25 μg/kg body weight per min (0.8 mg of nitroglycerin/ml 5% dextrose in water) and increased by 0.25 μg/kg per min increments every 5 min to a maximum of 1.5 μg/kg per min. This target dose is slightly higher than the average infusion rate used in patients with rest angina (25,26). In our experience, it is well tolerated and effective in patients with severe heart failure. The use of a fixed target dose stems from the fact that in this condition, blood pressure is often low even before nitroglycerin administration. Therefore, a decrease in blood pressure as an end point may not be as useful in monitoring clinical response in these patients as it is in those with rest angina.

The target dose was reached within the 1st hour in all patients. When a patient received nitroglycerin and N-acetylcysteine, both infusions were started together. Nitroglycerin infusion was started and increased as just described. N-acetylcysteine was given in the following manner: 50 mg/kg (in 100 ml 5% dextrose in water) in the first 30 min, 25 mg/kg per h (total of 200 ml 5% dextrose in water) for the next 2 h, then 100 mg/kg (in a total of 500 ml 5% dextrose in water) over the next 21.5 h. Maximal nitrate infusion rate could not be obtained until the 6th hour of infusion in five patients receiving N-acetylcysteine because of hypotension (systolic pressure <90 mm Hg). The 800 ml water given with the N-acetylcysteine was considered when the 1.5 liters/day fluid restriction was calculated.

Hemodynamic variables were measured and blood samples were drawn for hematocrit, hemoglobin, creatinine and blood urea nitrogen after 1, 6 and 24 h of nitroglycerin infusion. Plasma neurohumoral levels were only measured 1 and 6 h after the beginning of the infusion. Once the 24 h infusion was complete, patients treated with nitroglycerin alone received an increased rate of infusion to 7.0 μg/kg per h for 30 min and hemodynamic measurements were repeated. Patients treated with nitroglycerin plus N-acetylcysteine received a bolus injection of 50 mg/kg (in 100 ml 5% dextrose in water) of N-acetylcysteine given over 30 min and hemodynamic measurements were repeated. All infusions were then stopped.

Twenty-four hours later, all patients underwent the same protocol except for two differences: 1) patients who received only nitroglycerin received nitroglycerin plus N-acetylcysteine and vice versa, and 2) no neurohumoral samples were drawn during the second infusion.

Side effects. Two of the 13 patients did not complete the study. One patient had an allergic reaction to N-acetylcysteine and received two infusions of nitroglycerin alone. A second patient developed signs of thrombosis of the right subclavian vein during the second infusion. The first infusion with nitroglycerin alone was completed without problems, but the second infusion with nitroglycerin plus N-acetylcysteine was not completed. For these reasons, there were 14 infusions of nitroglycerin alone and 11 infusions of nitroglycerin plus N-acetylcysteine.

Total blood volume determination. Before the first infusion, total red blood cell volume and intravascular volume were determined by the use of the dilution principle. A 12 ml sample of blood was drawn from the arterial cannula and added to 2 ml A-C-D solution (Frosst) (10 ml solution contains 132 mg dextrose, 250 mg sodium citrate, 80 mg citric acid and 10 ml distilled water) in a sterile vial to which 1.85 mBq chromium-51 was added. The vial was gently shaken and incubated in a water bath at 37°C for 20 min. The

vial was then placed at room temperature and 50 mg ascorbic acid was added to stop the labeling process. Ten minutes later, 5 ml labeled blood was reinjected into the patient through the proximal port of the Swan-Ganz catheter. A period of 30 min was allowed to assure homogeneous distribution of the labeled erythrocytes in the vascular space, after which a blood sample was taken from the arterial cannula. The blood hematocrit level was determined for the injection mixture and for the patient's blood. Aliquots of 2 ml blood and 2 ml plasma obtained from the injection mixture and from the patient were then counted. Background activity on 2 ml aliquots of blood and of plasma taken before red cell labeling was also determined and subtracted from the appropriate samples to obtain the net number of counts.

The total red blood cell volume could then be determined by the following formula:

$$TGV = \frac{5[STD - PL_1(1 - HT_1)]}{PT - PL_2(1 - HT_2)},$$

where TGV = total red blood cell (globular) volume (ml), STD = counts/min in 2 ml blood from the injection mixture (standard), PT = net counts/min in 2 ml blood from the patient, PL_1 = counts/min in 2 ml plasma from the injection mixture, PL_2 = net counts/min in 2 ml plasma from the patient, HT_1 = injection mixture hematocrit and HT_2 = patient hematocrit.

Total blood volume (TBV) was calculated as:

$$TBV = \frac{TGV}{HT_2} \times \frac{1}{0.915},$$

where 0.915 represents the correction factor to convert venous hematocrit into total body hematocrit. When arterial samples were drawn, this correction factor was omitted. For each patient, it was assumed that the total red blood cell (globular) volume remained constant throughout the study and consequently served as a standard to measure the intravascular volume changes during the nitrate infusions. Vascular volume determinations could not be obtained in three patients because they had been exposed to radioactive diagnostic products in the month preceding the study.

Control patients to validate the technique. Seven patients with severe congestive heart failure (New York Heart Association class III or IV) had blood volume determined as inpatients (n = 3) or as outpatients (n = 4). These control patients continued their regular medications without any modification and had a strict fluid restriction of 1.5 liters/day (including the intravenous solution given during the 1st day of the study). Red blood cell labeling and blood sampling for hematocrit determination were done over 72 h at the same intervals as for the study patients. The hospitalized control patients remained at bed rest for the duration of the study, whereas the outpatient control subjects were admitted to the research outpatient clinic on 3 consecutive mornings and remained at bed rest at least 1 h before blood sampling and throughout the day. On the 1st day of the study, an infusion of 5% dextrose in water was given at a rate of 40 ml/h for 8 h to facilitate the labeling process and blood sampling.

Estimated mean total body radiation dose for blood volume determination in each patient was 20.8 ± 18.6 millirads.

Other methods and techniques. Arginine vasopressin, plasma renin activity and aldosterone were measured according to methods previously developed and reported by our laboratory (24). Atrial natriuretic peptide was measured by radioimmunoassay according to the technique of Wilson et al. (27). Arterial norepinephrine and epinephrine levels were measured according to the radioenzymatic assay of Peuler and Johnson (28). The normal values in our laboratory are as follows: plasma renin activity <2.5 ng/ml per h for a normal sodium diet, plasma aldosterone ≤25 ng/dl for a normal sodium diet, plasma vasopressin 0.5 pg/ml to 2.5 pg/ml for a plasma sodium level between 135 and 145 mEq/liter. Normal values for atrial natriuretic peptide are <30 pg/ml. Normal values for arterial norepinephrine are 170 ± 40 pg/ml and for epinephrine 35 ± 8 pg/ml. Hemodilution was measured as the percent change in hematocrit compared with baseline values.

Statistical analysis. Patients were divided in two groups according to whether they received nitroglycerin alone or nitroglycerin plus N-acetylcysteine. For each group, sequential measurements of hemodynamic variables, body weight, neurohormones, hematocrit and total blood volume were analyzed by a repeated measures analysis of variance followed, when a significant change was found, by the multiple comparison test of Newman-Keuls. Total blood volume measurements in the control group were analyzed by a repeated measures analysis of variance, followed by Dunnet's test. Total blood volume variation for the entire study group as a function of time was fitted by a rectangular hyperbola with the use of a simplex algorithm method (29). Statistical significance was accepted at $p < 0.05$. Unless otherwise stated, all values are mean values ± SE.

Results

The study patients had severe congestive heart failure and significant neurohumoral activation (Fig. 1 and 2). These patients also had mild renal failure (serum creatinine 1.47 ± 0.32 mg/dl and blood urea nitrogen 33 ± 2 mg/dl [mean ± SD]).

Hemodynamic and neurohumoral measurements. The overall hemodynamic effects of the 24 h nitroglycerin infusions were similar whether nitroglycerin was given alone or in combination with N-acetylcysteine. The same partial tolerance to nitroglycerin occurred with both treatment regimens. The only difference was that it took 6 h to reach

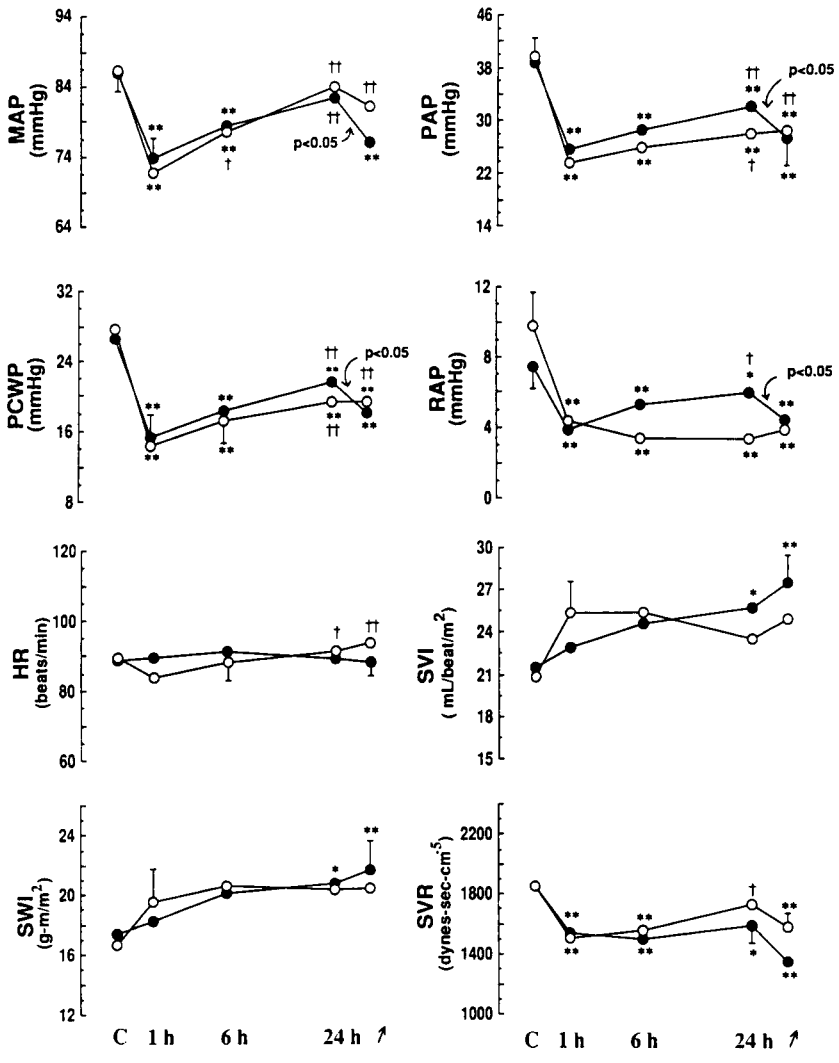


Figure 1. Hemodynamic changes caused by a 24 h infusion of nitroglycerin **(filled circles)** with and without N-acetylcysteine **(open circles)**. Values are indicated at control (C) and at 1, 6 and 24 h of infusion. The **arrow** at 24 h indicates values after increasing the rate of nitroglycerin infusion. N-acetylcysteine did not significantly alter the hemodynamic effects of nitroglycerin. Increasing the nitroglycerin infusion rate further decreased arterial and ventricular filling pressures. A bolus injection of N-acetylcysteine at the end of the infusion had no effect. For clarity, only the largest standard error of the mean for each group is indicated. *$p < 0.05$ and **$p < 0.01$ versus control and †$p < 0.05$ and ††$p < 0.01$ versus 1 h. HR = heart rate; MAP = mean arterial pressure; PAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; SVI = stroke volume index; SVR = systemic vascular resistance; SWI = stroke work index.

maximal nitroglycerin infusion rates in 5 of 11 patients receiving N-acetylcysteine because of hypotension, whereas all patients receiving only nitroglycerin reached maximal infusion rates after 1 h. After 1 h of infusion, mean arterial, pulmonary artery, pulmonary capillary wedge and right atrial pressures and systemic vascular resistance all decreased significantly, whereas there was no significant change in heart rate, stroke volume or stroke work index (Fig. 1), although stroke volume and stroke work index tended to increase in both groups. These decreases in arterial and ventricular filling pressures were accompanied by an increase in arterial epinephrine and plasma renin activity and a decrease in atrial natriuretic peptide (Fig. 2). There was no change in arterial norepinephrine, aldosterone or arginine vasopressin levels.

After 6 h of continuous infusion, no further hemodynamic changes occurred, except that mean arterial pressure tended to increase and this increase was significant in patients receiving N-acetylcysteine (Fig. 1). This increase might have been greater had a maximal nitroglycerin dose been achieved in all of these patients by 1 h of infusion. By 6 h of infusion, mean arterial, pulmonary artery, right atrial and pulmonary capillary wedge pressures and systemic vascular resistance all remained lower than control values (Fig. 1). Again, there was no change in heart rate, stroke work or stroke volume index, although stroke volume and stroke work index both still tended to be higher than baseline values. By 6 h of continuous infusion, arterial epinephrine levels had returned to baseline values, possibly because of less pronounced hypotension; however, plasma renin activity still remained elevated and atrial natriuretic peptide continued to be decreased (Fig. 2). There was no change in the levels of the other neurohormones (norepinephrine, aldosterone and arginine vasopressin).

After 24 h of continuous infusion, mean pulmonary artery, pulmonary capillary wedge and right atrial pressures were all still decreased compared with baseline measurements (Fig. 1). However, except for atrial pressure in the N-acetylcysteine group, all of these variables had increased compared with values after 1 h of infusion. By 24 h of

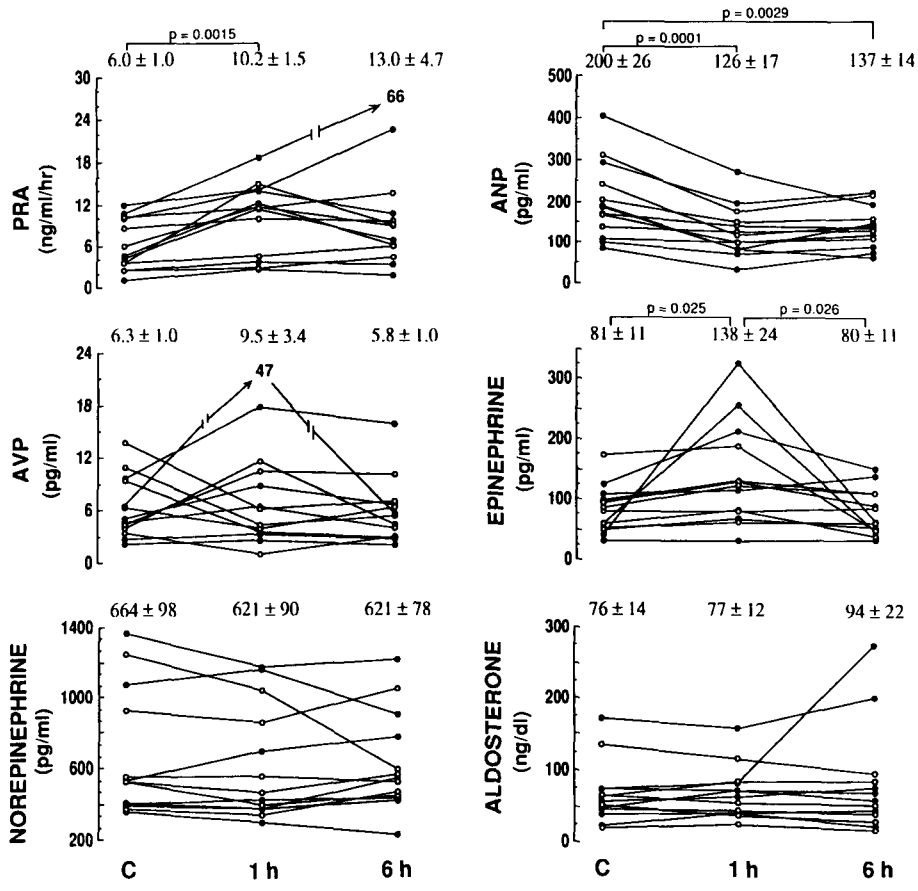


Figure 2. Neurohumoral changes during the first 6 h of nitroglycerin infusion. Arterial epinephrine increased after 1 h of infusion, then returned to the baseline value. Plasma renin activity (PRA) increased and atrial natriuretic peptide (ANP) decreased throughout the first 6 h of the infusion. **Filled circles** = patients with nitroglycerin alone (n = 7) and **open circles** = patients with nitroglycerin plus N-acetylcysteine (n = 6); p values are indicated when significant. AVP = arginine vasopressin.

continuous infusion, mean arterial pressure had returned to baseline values in both groups. Although stroke work index and stroke volume index increased and systemic vascular resistance decreased similarly in both groups, these changes were significantly different from baseline values only in the group taking nitroglycerin alone.

Increasing the nitroglycerin infusion rate from 1.5 to 7 μg/kg per min after 24 h in the group receiving only nitroglycerin caused a further decrease in mean pulmonary artery, pulmonary capillary wedge and right atrial pressures (Fig. 1). It also caused a decrease in mean arterial pressure but did not significantly alter other hemodynamic variables. Increasing the infusion rate of N-acetylcysteine (50 mg/kg bolus injection) did not significantly alter hemodynamic values.

Blood volume measurements. Throughout the 24 h infusion, hematocrit decreased and total blood volume increased (Fig. 3). These changes occurred despite fluid restriction and there was no change in mean body weight (day 0, 65.2 ± 8.7; day 1, 65.5 ± 8.5; day 2, 65.2 ± 8.6; and day 3, 65.4 ± 8.8 kg). By 1 h of continuous infusion, hematocrit decreased and total blood volume increased similarly in both groups, indicating that the fluid injected with the N-acetylcysteine had no bearing on these results.

Total blood volume continued to increase between 1 and 24 h of infusion in both groups. Both groups had a similar degree of hemodilution after 24 h of infusion (9.1 ± 4.3% for nitroglycerin alone and 8.7 ± 4.1% for nitroglycerin plus N-acetylcysteine). When both groups are considered together, this hemodilution resulted in an increase in intravascular volume of 745 ± 382 ml. When the percent changes in total blood volume of the individual patients are plotted as a function of time, data points are best fitted by a rectangular hyperbola (standard deviation of the equation 1.20×10^{-1}, sum of squared residuals 2.28×10^{-1}) (Fig. 4):

$$\text{Change in total blood volume} = \frac{0.141T}{T + 0.550\,h},$$

where T represents the time in hours. The coefficient in the numerator represents the maximal asymptotic value for the change in blood volume (14.1%). The second term in the denominator (0.550 h) represents the time at which half of the asymptote has been reached. There was no change in blood volume in our control patients (Fig. 5).

Discussion

Hemodynamic tolerance to nitroglycerin appears to be multifactorial. This study suggests that the development of hemodynamic tolerance to nitroglycerin in patients with congestive heart failure is multifactorial. As previously reported (1), a major mechanism of tolerance appears to be

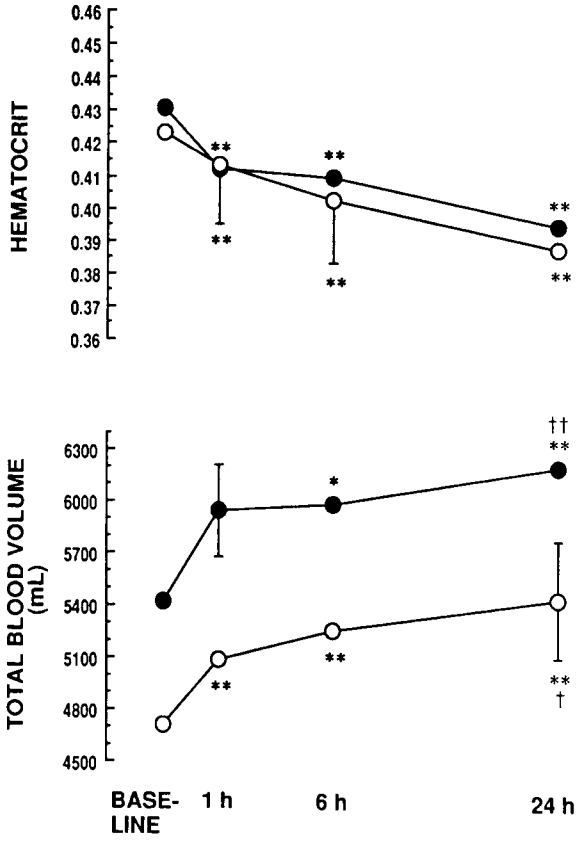


Figure 3. Changes in hematocrit and blood volume during nitroglycerin infusion. Hematocrit decreased and blood volume increased similarly in both groups. For clarity only the largest standard error of the mean for each group is illustrated. *$p < 0.05$ and **$p < 0.01$ versus control and †$p < 0.05$ and ††$p < 0.01$ versus 1 h. **Open circles** = patients who received only nitroglycerin (n = 11) and **filled circles** = patients who received nitroglycerin plus N-acetylcysteine (n = 8).

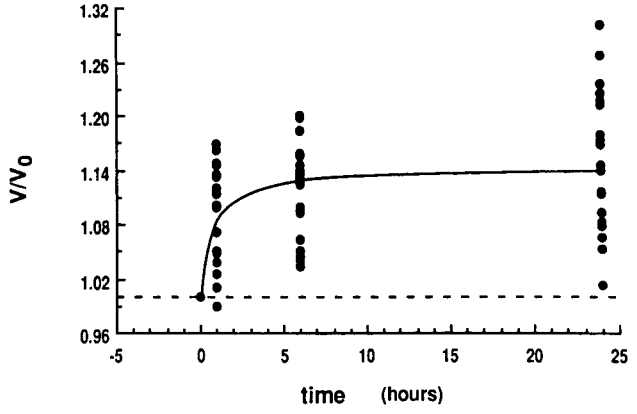


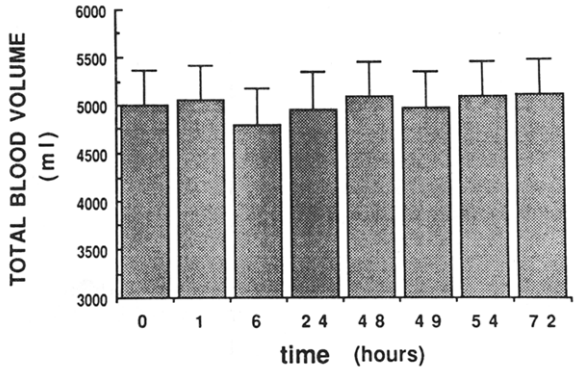
Figure 4. Assessment of time course and maximal variation in the total blood volume of patients receiving intravenous nitroglycerin. Data points were best fitted by a rectangular hyperbola; standard deviation = 1.20×10^{-1}; sum of squared residuals = 2.28×10^{-1}. The maximal asymptotic value for the variation of blood volume is 14.1%. V/V_0 represents the ratio of individual blood volumes over the baseline blood volumes. The **dotted line** represents a ratio of 1.

a loss of pharmacologic effect of the drug on vascular smooth muscle. However, in this study, the simultaneous infusion of N-acetylcysteine did not prevent the development of the partial hemodynamic tolerance that was observed with nitroglycerin alone. A second mechanism, a large fluid shift from the extravascular to the intravascular space, appears to be involved, especially during the 1st hour of infusion when this fluid shift is greatest. A third mechanism, neurohumoral activation, also seems to contribute to the development of tolerance to nitroglycerin.

Comparison with previous studies involving N-acetylcysteine. The most probable cause for the differences in results between this study and previous investigations (5,14) is the dosages of N-acetylcysteine and nitroglycerin used. N-acetylcysteine has been shown to potentiate the effects of nitroglycerin and increase arteriovenous nitroglycerin extraction (30–32). This reaction appears to be dose dependent, to occur largely in plasma and to result in nonspecific vasodilation whether or not nitrate tolerance exists (15). Both Packer et al. (5) and May et al. (14) used large acute doses of N-acetylcysteine (200 and 100 mg/kg) and large doses of nitroglycerin (6.4 μg/kg per min and 45 μg/min intravenously, respectively, plus large intracoronary doses [10 to 100 μg]) and it may be that such large circulating levels of these medications resulted in the extracellular conversion of organic nitrates to an active compound (15,33–35). The need for a combination of high circulating nitroglycerin and N-acetylcysteine levels for "apparent nitrate tolerance reversal" would certainly explain why Packer et al. (5) had such a brief (<90 min) beneficial effect with N-acetylcysteine.

Thus, the effects of N-acetylcysteine in their study may have been the result of an extracellular reaction between high circulating doses of nitroglycerin and N-acetylcysteine rather than intracellular smooth muscle cell sulfhydryl group repletion. This extracellular activation of nitrates to an

Figure 5. Blood volume measurements (±SEM) in seven control patients with stable heart failure over a 3 day period. There was no increase in blood volume.



active compound appears to occur with nitroglycerin but not with isosorbide dinitrate (33). This specificity may explain why Parker et al. (17) were unable to reverse tolerance to the beneficial effects of isosorbide dinitrate on exercise capacity in patients with angina. Alternatively, the dose of isosorbide dinitrate (30 mg) or of N-acetylcysteine (100 mg/kg) may have been too small to obtain reversal of tolerance.

It is possible that the dose of N-acetylcysteine used in this study was inadequate to replete intracellular sulfhydryl groups. However, if one considers the decrease in volume of distribution and hepatic clearance that occurs in heart failure (36,37), the dose used in this study was likely superior to that found effective in treating acetaminophen intoxication (38,39). Therefore, adequate circulating levels of N-acetylcysteine to provide sulfhydryl groups were likely present. In addition, the doses used in this study were equivalent if not larger than the doses used to decrease the incidence of myocardial infarction in patients with unstable angina (40). Finally, it is unlikely that an inadequate number of sulfhydryl group donors was the only reason for the lack of effect of N-acetylcysteine in our study because the bolus injection of N-acetylcysteine given at the end of the study had no effect.

One obvious difference between this study and previous investigations (5,14) is the length of time the N-acetylcysteine had been given before taking hemodynamic measurements. In our study, N-acetylcysteine was infused for 24 h to prevent tolerance, whereas in the previous investigations, N-acetylcysteine was given acutely to reverse tolerance. Although unlikely because of its continued efficacy in treating acetaminophen overdose, it is possible that a certain tolerance to the beneficial effects of N-acetylcysteine occurs with time and that a second mechanism of nitrate tolerance occurs, perhaps a change in enantioselectivity (16).

The degree of tolerance achieved in this study was less than that described in previous studies (5,6). This difference could be related to the shorter period of infusion (24 versus 48 h) and may at least partially explain why N-acetylcysteine did not prevent the development of tolerance. The contribution of pharmacologic tolerance during this relatively short period of infusion may have been insufficient to document a significant effect of N-acetylcysteine. Had the duration of infusion been longer and tolerance more complete, N-acetylcysteine might have been more effective. For all practical purposes, however, N-acetylcysteine does not appear to be a clinically useful agent in preventing early tolerance to nitroglycerin.

Role of fluid shift. Our results suggest that a second mechanism, a fluid shift from the extravascular to intravascular space, may play a role in the development of nitrate tolerance. This fluid shift would result in an increased intravascular volume, which would counterbalance the vasodilating effects of nitroglycerin. An increase in intravascular volume has already been shown to be the major cause of tolerance to nearly all vasodilators used to treat hypertension (20,21) and there is no reason to believe that an increase in intravascular volume as large as the one documented in this study did not influence our hemodynamic findings.

The large increase in intravascular volume occurred despite strict fluid restriction (1.5 liters/day) before and during the study. The 1.5 liter fluid restriction included both oral and intravenous liquids, when extra intravenous liquids were given, oral intake was reduced correspondingly. It is unlikely that such an increase in intravascular volume was the result of intravenous fluid administration itself because the thermodilution catheter was inserted and intravenous infusions were started the evening before beginning the study. Also, between nitroglycerin infusions, hematocrit and blood volumes returned toward baseline values despite continued intravenous fluid administration. No change in body weight occurred throughout the study and the major portion of the increase in intravascular volume occurred so quickly that a shift of fluid from the extravascular to intravascular space is the most likely explanation for our findings.

The reason for this fluid shift remains speculative, but it may simply be the result of modification of Starling forces in the capillary bed caused by preferential vasodilation of veins and venules as opposed to arteries and arterioles (3,4). Intravascular hydrostatic forces favoring extravasation of fluid from the capillaries to the extravascular space would be decreased, thus favoring an increase in intravascular volume. The end result would be similar to what happens when a patient with heart failure moves from the standing to the supine position. Because fluid balance and body weight remained stable throughout the study, the use of fluid loading or diuretic drugs to further study the effects of fluid shifts on nitrate tolerance would have been difficult to interpret because these interventions would have resulted in an increase or decrease in total body fluid and by themselves would have modified all hemodynamic variables.

The fluid shift occurred mostly during the 1st h of the infusion. This may be one reason why increasing perfusion rates of nitroglycerin over 1 or 2 h is better tolerated than starting the same dose suddenly. Intravascular volume also continued to increase, but to a lesser extent, between h 1 and h 24 of nitroglycerin infusion. A fluid shift may therefore have played a role in the development of the partial tolerance to nitroglycerin that was observed in this study, and may help explain why N-acetylcysteine was unsuccessful in preventing it.

Role of neurohormones. During the nitroglycerin infusion, there was an increase in arterial epinephrine and plasma renin activity and a decrease in atrial natriuretic peptide levels. This decrease in atrial natriuretic peptide levels, which occurred despite volume expansion, may be explained by the lower atrial pressures recorded throughout the infusion. Neurohumoral activation may have been useful in maintaining arterial pressure during the first hours of

infusion. However, because epinephrine decreased to baseline values between h 1 and h 6 of the infusion and other neurohormones did not change during this period, it is unlikely that neurohumoral activation caused the observed loss of effect of nitroglycerin. What long-term effects an increase in plasma renin activity and decrease in atrial natriuretic peptide would have in these patients are uncertain, but there is good reason to believe that it would lead to volume expansion, an increase in systemic vascular resistance and a loss of the initial beneficial effects of nitroglycerin. In a previous study (5), intravenous nitroglycerin was found to cause a sustained (48 h) increase in plasma renin activity and an increase in body weight. However, two other studies (6,18) did not report such findings, and the role of long-term reflex neurohumoral activation in the development of tolerance remains to be determined.

This study may have underestimated the role of neurohumoral changes in the development of nitroglycerin tolerance for two reasons. 1) It was done with the patient in the supine position and postural hemodynamic and neurohumoral changes caused by nitroglycerin were not assessed. Measurements in patients in the upright position (41) or during exercise (42) may have brought out differences in neurohumoral activation not seen at rest. 2) As a result of strict control of sodium and water intake during this study, the tendency toward sodium and water retention was minimized, such that a neurohumorally mediated tendency toward volume expansion may have been missed. Longer follow-up study would have been necessary to fully appreciate the contribution of reflex neurohumoral activation to long-term nitrate tolerance.

Conclusions. This study suggests that the development of tolerance to nitroglycerin is multifactorial. A loss of pharmacologic effect of nitroglycerin on smooth muscle has been well described, but in the present study, the simultaneous infusion of N-acetylcysteine in doses shown effective in treating acetaminophen intoxication did not prevent the development of the partial hemodynamic tolerance that was observed with nitroglycerin alone. These results do not support a practical role for N-acetylcysteine in preventing early tolerance to nitrates in congestive heart failure. A second mechanism, a fluid shift from the extravascular to the intravascular space, seems to contribute to hemodynamic tolerance to nitroglycerin, especially during the 1st hour of infusion. A third mechanism, neurohumoral activation, may also be involved in early tolerance to nitroglycerin. Because of the nature of this study, however, the role of neurohormones in the development of nitroglycerin tolerance may have been underestimated.

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